REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
17-05-2006	Final Technical Report	March 1, 2002 to Dec. 31, 2005

4. TITLE AND SUBTITLE
Preliminary Studies Examining Near Ultraviolet Fluorescence and Raman Spectroscopy for Tissue Interrogation of Shock

5a. CONTRACT NUMBER

5b. GRANT NUMBER
N00014-02-1-0344

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Terner, James

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Virginia Commonwealth University
P.O.Box 0568
800 East Leigh St. Suite 113
Richmond, VA 23298-0568

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research
Casualty Care and Management
875 North Randolph Street
Arlington, VA 22203-1995

10. SPONSORING/MONITOR'S ACRONYM(S)
ONR

11. SPONSORING/MONITORING REPORT NUMBER

12. DISTRIBUTION/AVAILABILITY STATEMENT
Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Non-invasive methods for the measurement of tissue oxygen saturation have long been sought for the detection of impending shock and the adequacy of resuscitation. Current methods such as those that require insertion of a catheter into the pulmonary artery or superior vena cava have attendant risks to the patient. Gastric tonometry, though considered to be minimally invasive, still requires insertion of a nasogastric tube into the stomach. We have found that we can obtain resonance Raman and fluorescence signals from the sublingual surface of the tongue in laboratory animals in a non-invasive manner, that correlate with lactate and oxygenation measurements of blood withdrawn by central venous catheterization. The resonance Raman measurements monitor hemoglobin oxygenation. The fluorescence measurements monitor NADH levels. In addition to being noninvasive the measurements are selective against contaminating signals from myoglobin in deep tissue. Even with low powered laser excitation the resonance Raman signals are quite strong, and both methods are adaptable for use with portable fiber optic components.

15. SUBJECT TERMS
Traumatic Shock, Septic Shock, Hemorrhage, Resonance Raman Spectroscopy

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19b. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Dr. James Terner
				5	19b. TELEPHONE NUMBER *(Include area code)* (804) 828-7500

FINAL REPORT

GRANT TITLE: Preliminary Studies Examining Near Ultraviolet Fluorescence and Raman Spectroscopy for Tissue Interrogation of Shock

PRINCIPAL INVESTIGATOR: James Terner

INSTITUTION: Virginia Commonwealth University

GRANT NUMBER: N00014-02-1-0344

AWARD PERIOD: March 1, 2002 to December 31, 2005

REPORTING PERIOD: March 1, 2002 to December 31, 2005

OBJECTIVE:

Our objectives have been to develop non-invasive methods for the assessment of oxygen saturation of tissue. Presently available methods are risky, unpleasant for the patient, and are difficult to implement. The most commonly used methods require insertion of a catheter into a major vessel near the heart, or insertion of a nasogastric tube. Our goals are to develop non-invasive methods using portable instrumentation that can be adapted for use in the field or clinic.

APPROACH:

Our approach has been to develop methods for measuring oxygen saturation levels with resonance Raman spectroscopy, and NADH levels via fluorescence, utilizing portable instrumentation. While miniaturized lasers have now been available for some time, the introduction of miniaturized fiber optic spectrometers is an offshoot of the development of fiber optic systems for the telecommunications industry. These miniaturized optical components now make it practical to implement spectroscopic measurements external to a laboratory setting. Such measurements were previously limited to hard wired laboratories due to requirements for electrical power, plumbing, and cryogenic cooling of high performance scientific instrumentation.

ACCOMPLISHMENTS (for the entire 3-year period):

Fluorescence and Raman measurements are generally performed with the same type of instrumentation: an excitation source such as a laser, and a spectrometer to provide a readout of intensity of scattered light vs. wavelength. Raman signals tend to be weak and occur close to the excitation line. Fluorescence is much stronger and usually occurs further away from the excitation wavelength than Raman signals. Unfortunately Raman and fluorescence measurements tend to compete with one another, in the sense that in cases where Raman signals and strong fluorescence occur at the same wavelength, the Raman signals will be drowned out. By contrast, in the work described here, we have identified an excitation wavelength region that when used on tissue beds in the oral cavity, the Raman and fluorescence signals are well separated, and minor fluorescence interference does not present a problem to the acquisition of Raman signals.

Since Raman signals are much more specific than fluorescence, our initial work has been concentrated in the area of Raman spectroscopy. Moreover, such specificity has spawned a new industry that markets portable Raman spectrometers. Most systems sold today utilize near-infrared excitation, with a popular wavelength being 785 nm. Near-infrared excitation is widely considered to be the wavelength of choice for avoiding fluorescence problems. By exciting below the energies of electronic absorptions, fluorescence emission can be avoided from the samples themselves, or from the impurities contained therein, that would otherwise obscure Raman signals generated by visible excitation. Commercial systems that do not utilize near-infrared wavelength generally use 532 nm, and capitalize on existing applications that have already been developed for the common 532 nm Nd:YAG laser line.

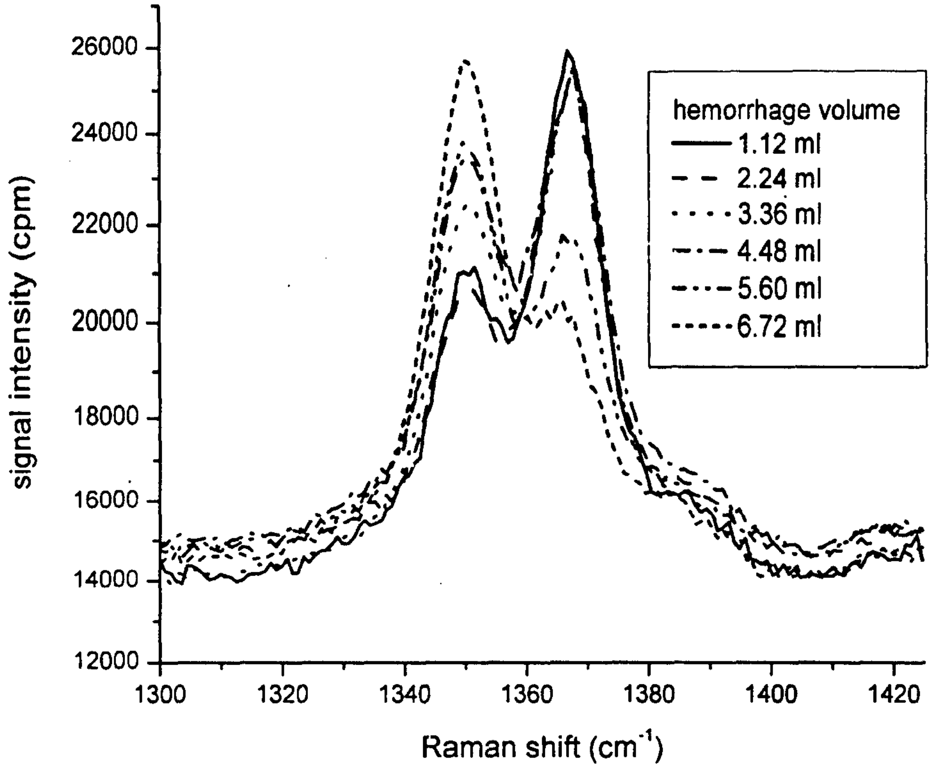

Figure 1. Resonance Raman spectra obtained from the sublingual surface of a rat tongue under controlled hemorrhage. 5 mW 406.7 nm excitation.

Though we initially investigated 532 nm, 785 nm and other wavelengths in the visible and near-infrared, we obtained surprising results in another wavelength region. We found that the best Raman signals for the application described in this report were obtained with deep violet excitation in the vicinity of 405 nm. This was unexpected since 405 nm is normally expected to induce strong fluorescence. By contrast we found that 405 nm excitation gives rise to remarkably strong resonance Raman signals from hemoglobin, with the bulk of fluorescence emission occurring at longer wavelengths. Equivalently strong signals are not obtained with near-infrared excitation due to loss of the opportunity for resonance enhancement. Furthermore, a phenomenon known as the ν^4-dependence is manifested as markedly stronger Raman signals at

shorter as opposed to longer excitation wavelengths. We found that the resonance enhancement effect combined with the v^4-dependence allowed us to utilize only 5 mW of 405 nm excitation, as compared with the several hundred milliwatts commonly utilized with near-infrared excitation. Thus the problem of intense local heating of tissue with near-infrared excitation is avoided.

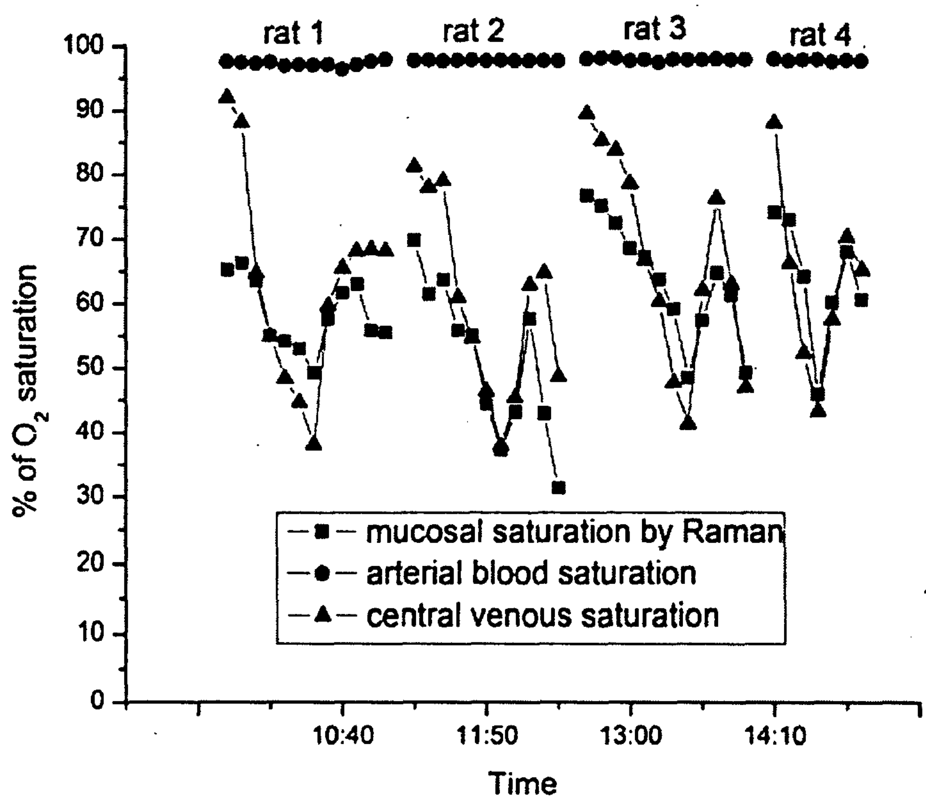

Figure 2. This diagram shows that resonance Raman measurements of the oxygen saturation of mucosal oral tissue (sublingual surface of the tongue), shown in Fig. 1, correlate with venous rather than arterial blood saturation.

An example of our resonance Raman results is given in Figure 1 for a set of experiments done with rats. Resonance Raman signals were obtained from the sublingual surface of the tongue under conditions of controlled hemorrhage. Resonance Raman peaks at 1375 cm^{-1} (oxyhemoglobin) and 1355 cm^{-1} (deoxyhemoglobin) are well separated, and the relative peak heights varied with the extent of hemorrhage and resusucitation. Simultaneously with the Raman measurements, blood was withdrawn from a central venous catheter for comparison purposes. The relative intensities of the Raman bands were found to track with oximeter measurements of the withdrawn blood. The correlation of the Raman measurements with central venous saturation determined by oximetry is shown in Figure 2. The results show that the Raman measurements correlate with the oxygenation of central venous blood rather than the oxygenation of arterial blood.

SIGNIFICANCE:

The determination of the saturation of arterial blood via pulse oximetry is now commonplace. However there is no comparable method for the determination of the saturation of venous blood or tissue. As we have illustrated in Figure 2, the saturation of arterial blood can be close to 100% despite the presence of a life threatening situation such as hemorrhage. The normal oxygen saturation of venous blood is generally 70%. This percentage is generally lowered with hemorrhage, and can be raised with sepsis. Such conditions are difficult to detect through the traditional vital signs such as blood pressure, pulse rate or body temperature, or through patient history. There is thus a need to reliably measure venous blood oxygen saturation to serve as a guide to the implementation of proper therapy. Such measurements could also reduce the number of unnecessary blood transfusions and the resulting problems such as blood shortages, infections, and reperfusion injury etc. that unnecessary blood transfusions cause. Once therapy is instituted, venous blood saturation measurements would provide an indication as to when resuscitation efforts have been effective and the patient can be safely discharged.

The methods we have developed are adaptable to the use of fiber optics and portable spectrometer systems, with the laser power requirements at 405 nm being quite modest. The introduction of 405 nm laser diodes has been relatively recent. We have noticed that 405 nm laser diodes have longevity problems at the present time. However improvement of the technology is being driven by the short wavelength requirements for new high density DVD players.

PUBLICATIONS, ABSTRACTS, TECHNICAL REPORTS, PATENTS, AND AWARDS (last 12 months):

"Hemoglobin oxygen saturation measurements using resonance Raman intravital microscopy" I.P. Torres Filho, J. Terner, R.N. Pittman, L.G. Somera, and K.R. Ward (2005) Am. J. Physiol. (Heart Circ. Physiol.), 289: H488–H495.

"Resonance Raman spectroscopy: A new technology for tissue oxygenation monitoring" Kevin R. Ward, Ivo Torres Filho, Robert W. Barbee, Luciana Torres, Mohamad H. Tiba, Penny S. Reynolds, Roland N. Pittman, Rao R. Ivatury, and James Terner; Crit Care Med 2006, 34, 792-800

United States Patent Application 20040039269 "Use of ultraviolet, near-ultraviolet and near infrared resonance Raman spectroscopy and fluorescence spectroscopy for tissue interrogation of shock states, critical illnesses, and other disease states" K.R. Ward, R.W. Barbee, J. Terner, R. Ivatury and F.M. Hawkridge